254

Braverman A[1], Ruggieri M[1]
1. Temple University School of Medicine

BLADDER HYPERTROPHY AND DEVERNATION INDUCE A SELECTIVE DEFICIT IN M3 RECEPTOR PROTEIN BUT NOT M3 RECEPTOR RNA

Aims of Study

Previous studies in the rat showed that experimental pathologies inducing bladder hypertrophy alter the muscarinic receptor subtype mediating contraction from M_3 towards M_2. In addition, the density of the M_2 receptor protein increased with hypertrophy while the density of the M_3 receptor decreased. We quantified mRNA for M_1 through M_5 transcripts to determine whether the changes in M_2 and M_3 mRNA are reflected by changes in protein concentration.

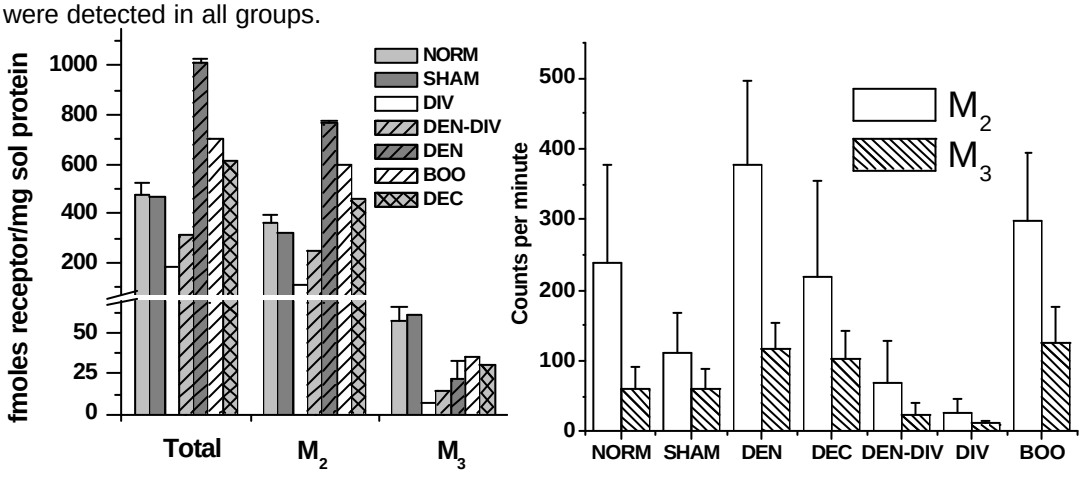
Methods

The density of M_2 and M_3 receptor protein was measured by subtype selective immunoprecipitation (IPPT). Transcripts for M_1-M_5, and two housekeeping genes, L32 and GAPDH, were quantified using a multiplex ribonuclease protection assay (RPA) such that all 7 transcripts were quantified from a single sample of RNA in a single lane of an electrophoresis gel. To separate bladder denervation from its associated hypertrophy, the following experimental groups were studied:

- major pelvic ganglion electrocautery (DEN, n=8) which results in both bladder denervation and hypertrophy,
- major pelvic ganglion electrocautery with bilateral ureteral diversion (DEN-DIV, n=8) which results in denervation without bladder hypertrophy,
- bladder outlet obstruction to the size of a 21 gauge syringe needle (BOO, n=5) which results in hypertrophy and perhaps some degree of denervation,
- major pelvic ganglion decentralization (DEC, n=6) which results in hypertrophy but is different than DEN in that the neural connections from the MPG to the bladder remain functionally intact,
- bilateral ureteral diverted (DIV, n=9) which results in atrophy,
- normal (n=7), and
- sham (n=7).

Results

Total and M_2 receptor protein density was decreased in the DIV and DIV-DEN groups and increased in the hypertrophied groups (DEN, BOO and DEC). M_3 receptor protein concentration was decreased in all groups except BOO. The receptor transcripts were not normalized to the housekeeping genes since the density of the transcripts for the housekeeping genes varied widely between groups. Receptor transcripts were expressed per 20 µg total RNA which was constant for each sample. Transcripts for all 5 receptor subtypes were detected in all groups.



The densities of M_1, M_4, and M_5 were much lower than for the M_3 subtype. There were more M_2 receptor transcripts than all the others, consistent with M_2 protein determinations. M_2 transcripts were significantly increased in DEN compared to sham, DEN-DIV and DIV. Surprisingly, M_3 transcripts were also significantly increased in DEN compared to sham, DEN-DIV, and DIV and increased in BOO bladders compared to DEN-DIV and DIV. No differences in M_1, M_4, or M_5 transcript densities were found. The M_2 receptor protein density was significantly correlated with the M_2 receptor transcript densities ($R=0.95$, $p=0.0013$) while there was no significant correlation between the density of M_3 receptors and M_3 transcripts ($R=0.16$, $p=0.73$). The denervated and decentralized bladders had a decrease in the density of M_3 receptor protein.

Conclusions

For the M_2 receptor, changes in mRNA density correspond to changes in protein density, conversely, changes in M_3 receptor mRNA density are not reflected by changes in M_3 protein density. It appears that with increasing hypertrophy there is an increase in the density of the M_2 receptor subtype and consequently the M_2 receptor mRNA. Thus the clinical use of anticholinergics that selectively target the M_2 receptor subtype may not only result in less M_3 mediated side effects, such as dry mouth, but also more effectively suppress the M_2 mediated urinary bladder hyperactivity.